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The Transcription Factor Yin Yang 1 Is Essential for Oligodendrocyte Progenitor Differentiation

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SUMMARY

The progression of progenitors to oligodendrocytes requires proliferative arrest and the activation of a transcriptional program of differentiation. While regulation of cell cycle exit has been extensively characterized, the molecular mechanisms responsible for the initiation of differentiation remain ill-defined. Here, we identify the transcription factor Yin Yang 1 (YY1) as a critical regulator of oligodendrocyte progenitor differentiation. Conditional ablation of *yy1* in the oligodendrocyte lineage in vivo induces a phenotype characterized by defective myelination, ataxia, and tremor. At the cellular level, lack of *yy1* arrests differentiation of oligodendrocyte progenitors after they exit from the cell cycle. At the molecular level, YY1 acts as a lineage-specific repressor of transcriptional inhibitors of myelin gene expression (Tcf4 and Id4), by recruiting histone deacetylase-1 to their promoters during oligodendrocyte differentiation. Thus, we identify YY1 as an essential component of the transcriptional network regulating the transition of oligodendrocyte progenitors from cell cycle exit to differentiation.

INTRODUCTION

During development, oligodendrocytes derive from precursor cells residing throughout the neural axis (Pringle and Richardson, 1993; Noll and Miller, 1993; Hall et al., 1996; Miller, 2005; Richardson et al., 2006). Their maturation into myelin-forming cells is a complex event that requires exit from the cell cycle and the activation of a transcriptional program leading to expression of myelin genes. It was originally proposed that oligodendrocyte progenitor differentiation is intrinsically regulated by a "timing" mechanism that links the number of cell divisions to growth arrest and the initiation of differentiation (Temple and Raff, 1986). This mechanism was regulated by mitogens (Calver et al., 1998) and molecularly characterized by the progressive accumulation of cell cycle inhibitors (Durand et al., 1997). It was shown that genetic ablation of cell cycle regulators impaired differentiation of progenitors into oligodendrocytes and promoted the persistence of a proliferative state (Casaccia-Bonnefil et al., 1997; Durand et al., 1998). However, overexpression studies with viral vectors expressing cell cycle inhibitors did not induce differentiation of progenitors, even though cells arrested at the G1/S transition (Tikoo et al., 1998; Tang et al., 1999; Ghiani and Gallo, 2001). Together these studies suggested that cell cycle exit was necessary but not sufficient to induce differentiation of progenitors into oligodendrocytes. Because oligodendrocyte differentiation is dependent on the bioavailability of transcriptional activators and the corresponding decrease of transcriptional inhibitors (Wegner 2000; Stolt et al., 2002; Gokhan et al., 2005; Ligon et al., 2006; Marin-Husstege et al., 2006; Liu et al., 2006), in this study we investigate the mechanisms at the interface between cell cycle exit and the initiation of a transcriptional program of differentiation.

Previous studies from our group demonstrated that global deacetylation of histone H3 is one of the first events detected during this transition. Pharmacological inhibitors of histone deacetylases (i.e., HDACs) prevented the expression of myelin genes in vitro (Marin-Husstege et al., 2002) and developmental myelination in vivo (Shen et al., 2005) and suggested that histone deacetylation was necessary for the initiation of the transcriptional program leading to myelin gene expression.

To identify possible molecular links between histone deacetylation and transcriptional regulation of myelin genes, we screened the promoters of genes regulated by TSA, an inhibitor of HDAC activity during oligodendrocyte differentiation, for the presence of common binding motifs, using Gene2Promoter software (Genomatix Software GmbH, Munich). This analysis revealed the presence of the consensus sequence NNCCATNN (Shrivastava and Calame, 1994; Yant et al., 1995) for the transcription factor Yin Yang 1 (YY1) in the promoter region of over 30% of the genes downregulated by histone deacetylation during oligodendrocyte differentiation. YY1 is a zinc-finger

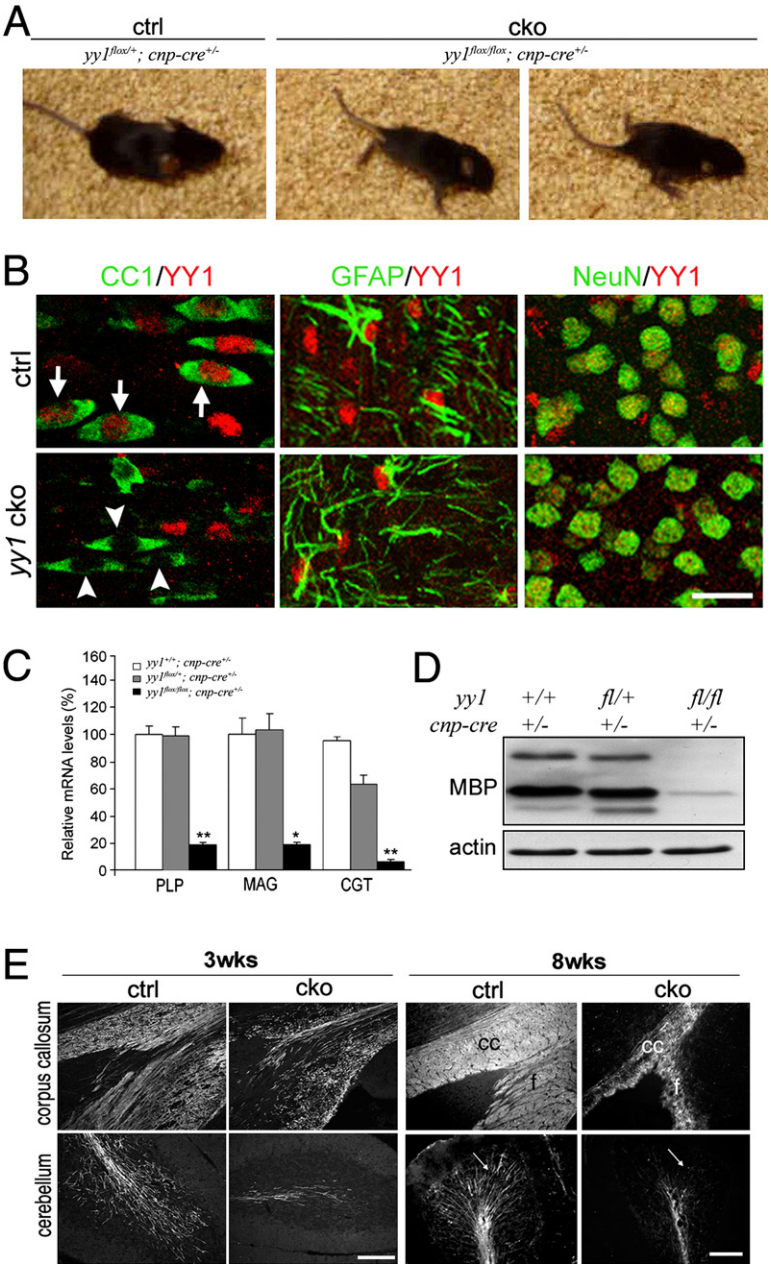

Figure 1. Conditional Ablation of YY1 in the Oligodendrocyte Lineage in Mice Results in a Trembling Phenotype

(A) Conditional *yy1* knockout mice (cko), obtained by crossing $yy1^{flox/flox}$ with *Cnp-cre* mice, displayed progressive tremor, ataxia, and head wobbling starting from the second postnatal week. Examples of abnormal posturing and gait in the mutant mice (cko) at postnatal day 18 (p18) are shown in comparison with control siblings (ctrl).

(B) Double immunostaining of cells in white matter tracts of p18 mouse sections stained for YY1 (red) and cell-specific markers (green): CC1 for oligodendrocytes, GFAP for astrocytes, and NeuN for neurons. Note the specific deletion of YY1 in oligodendrocytes. Scale bar, 20 μm.

(C) Quantitative real-time PCR of brain RNA from wild-type ($yy1^{+/+}$;$cnp\text{-}cre^{+/-}$), heterozygotes ($yy1^{flox/+}$;$cnp\text{-}cre^{+/-}$), and conditional knockout ($yy1^{flox/flox}$;$cnp\text{-}cre^{+/-}$) mice at p14. The levels of the indicated transcripts are normalized to GAPDH, and the mRNA control levels are arbitrarily set as 100. The bar graph indicates decreased myelin gene transcripts in *yy1* cko mice (black), but not in heterozygous siblings (gray) compared to controls (white) $^{*}p < 0.05$, $^{**}p < 0.01$. Error bars indicate mean ± SD.

(D) Western blot analysis of protein lysates from the cortex of p14 mice revealed decreased myelin basic protein (MBP) expression in the mutants. β-actin serves as loading control.

(E) Brain sagittal sections stained for PLP (3wks) and for MBP (8wks) reveal fewer myelinated fibers in the corpus callosum (cc), fornix (f), and cerebellum of *yy1* cko mice compared to controls (ctrl). Scale bar, 100 μm.

protein with the ability to bind DNA (Seto et al., 1991) and function as a chromatin modifier (Thomas and Seto, 1999; Liu and Shi, 2005). Genetic ablation of YY1 in mice resulted in peri-implatation lethality and suggested a role for YY1 in cell proliferation and differentiation (Donohoe et al., 1999).

Here we present an in vivo analysis of *yy1* conditional knockout (cko) mice in the oligodendrocyte lineage, and in combination with an integrated in vitro approach, we define YY1 as a molecular regulator of the transition from progenitors into myelinating oligodendrocytes.

RESULTS

Conditional Ablation of YY1 in the Oligodendrocyte Lineage Impairs Myelination

To assess the functional role of YY1 in oligodendrocyte differentiation in vivo, we generated *yy1* cko mice by employing a Cre/lox strategy by crossing $yy1^{flox/flox}$ mice (Affar et al., 2006) with a *Cnp-cre* line expressing the recombinase Cre from the oligodendrocyte lineage-specific *Cnp1* promoter (Lappe-Siefke et al., 2003). The resulting homozygous $yy1^{flox/flox}$;$cnp\text{-}cre^{+/-}$ mice (*yy1* cko; Figure 1A) appeared to be normal at birth but developed

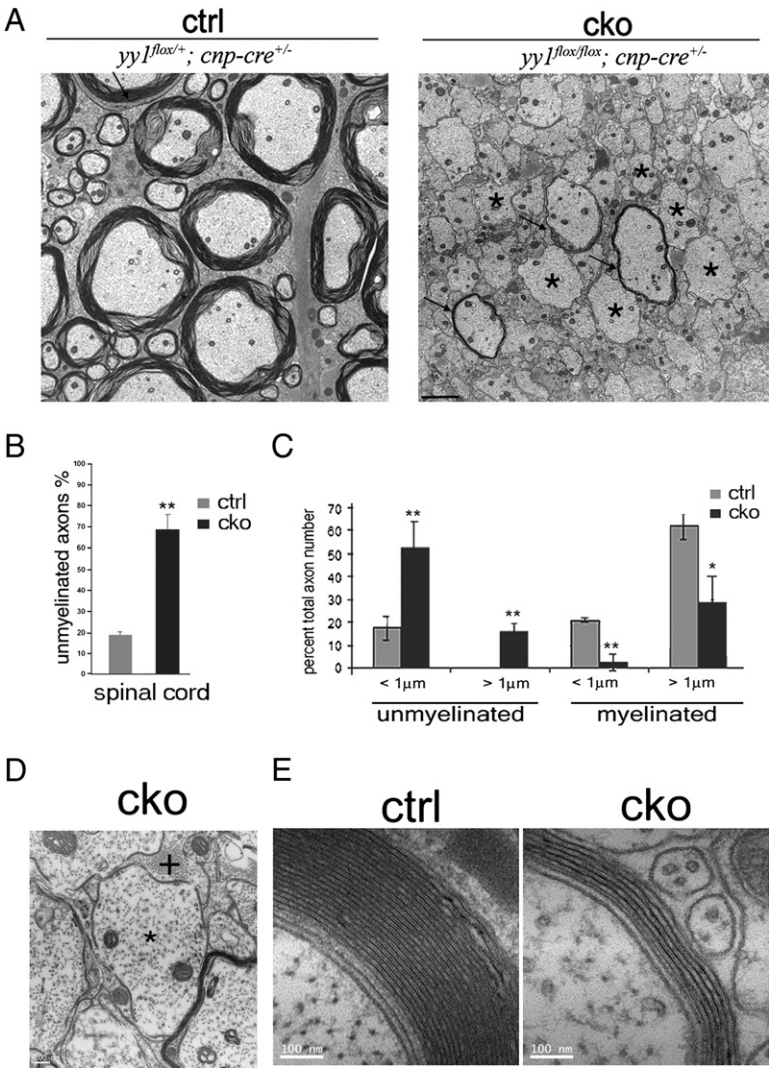

Figure 2. Defective Myelination in the Spinal Cord of *yy1* Conditional Knockout Mice

(A) Ultrastructural analysis of spinal cord sections shows lack of myelin (asterisks) and thinner myelin sheaths (arrows) in p18 *yy1* cko mice compared to controls. Scale bar, 2 µm.

(B) Bar graph shows increased percentage of unmyelinated axons relative to total number of axons in the spinal cord of *yy1* cko mice (black) compared to controls (gray).

(C) Bar graph shows the relative distribution of unmyelinated and myelinated axons in relation to axonal diameter in the spinal cord of *yy1* cko mice (black) and controls (gray) at p18. *$p < 0.05$; **$p < 0.01$. Error bars indicate mean ± SD.

(D) Representative EM picture of a large *yy1* cko spinal cord axon (diameter > 1.5 µm) that lacks myelin (*) and is in contact with an astrocytic process (+). Scale bar, 200 nm.

(E) Representative EM pictures of myelinated axons of equivalent diameter in control and *yy1* cko siblings. Scale bar, 100 nm.

shaking, ataxia, tremor, and head wobbling by 14 days of age (see Movie S1 in the Supplemental Data available with this article online) that persisted throughout adulthood. The cell-specific ablation of the *yy1* gene in cells of the oligodendrocyte lineage was confirmed by double immunofluorescence using antibodies against YY1 and against markers for different cell types: CC1 for oligodendrocytes, GFAP for astrocytes, and NeuN for neurons (Figure 1B). The lack of YY1 immunoreactivity in $CC1^+$ oligodendrocytes, but not in other cell types, indicated that the gene was efficiently excised in this lineage, although it was still expressed in astrocytes and neurons. Heterozygote $yy1^{flox/+}$;$cnp\text{-}cre^{+/-}$ mice did not show behavior abnormalities and were undistinguishable from wild-type mice. Because tremor and ataxia in *yy1* cko mice were highly reminiscent of the shaking phenotype described in myelin-deficient mouse mutants (Nave, 1994; Griffiths, 1996), we asked whether lack of YY1 inhibited myelin formation. Quantitative RT-PCR revealed 80% lower levels of transcripts for proteolipid protein (*plp*), myelin-associated glycoprotein (*mag*), and ceramide-galactosyl transferase (*cgt*) in the brains of p18 *yy1* cko mice compared to $yy1^{+/+}$;$cnp\text{-}cre^{+/-}$ controls (Figure 1C). Decreased myelin proteins were also detected by Western blot analysis and immunohistochemistry in the CNS of homozygous *yy1* cko mice, but not in heterozygous mice (Figures 1D and 1E). To determine whether this defective myelin gene expression persisted through adulthood, we analyzed the brain (Figure 1E) and spinal cord (data not shown) of adult cko mice and control siblings. Defective myelination was still detected in 8-week-old *yy1* mutant mice, thereby indicating the lack of compensatory mechanisms.

Defective myelination was confirmed by electron microscopy of the spinal cord, which revealed a 70% reduction of myelinated axons in *yy1* cko mice compared to controls (Figures 2A–2C). The few myelinated axons in *yy1* cko mice were characterized by thinner myelin sheaths (Figures 2D and 2E) and higher g ratios (0.892 ± 0.045) compared to control siblings (0.774 ± 0.047,

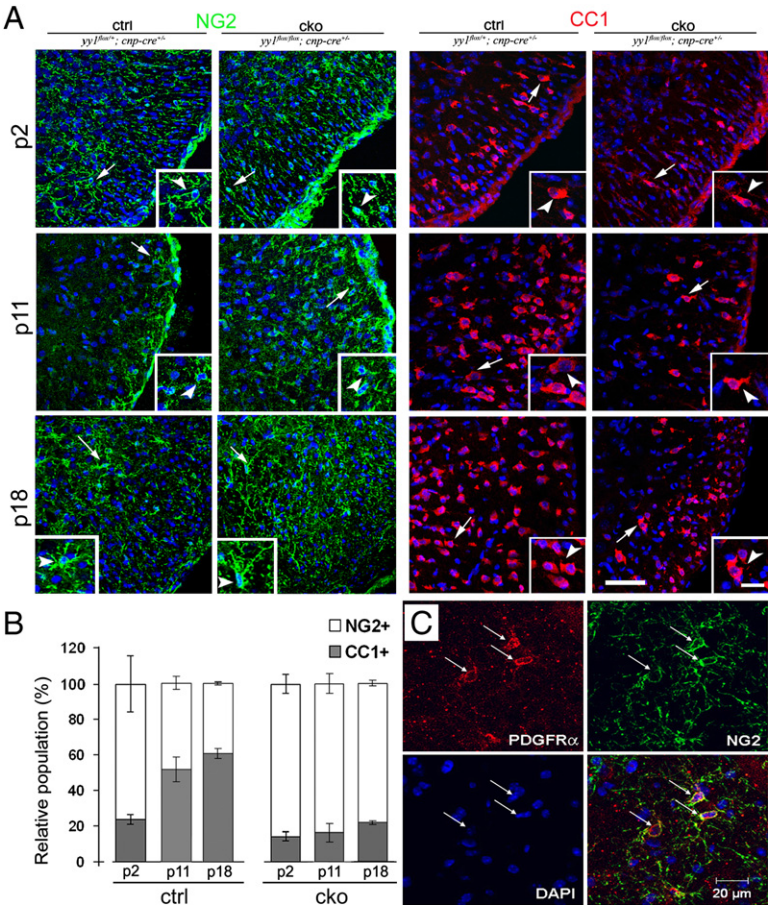

Figure 3. Impaired Oligodendrocyte Progenitor Differentiation in the Spinal Cord of *yy1* Conditional Mutants

(A) Confocal image of lumbar spinal cord sections from controls (ctrl) and *yy1* cko mice at postnatal days 2, 11, and 18, stained with antibodies specific for NG2 (green) to identify progenitors, CC1 (red) to identify oligodendrocytes, and DAPI (blue) to counterstain nuclei. Optical sections (Z, 1.0 μm; X, 12 μm) of confocal epifluorescence images were sequentially acquired, and LSM software was used to merge images. Examples of $NG2^+/DAPI^+$ and $CC1^+/DAPI^+$ cells selected for counting are shown in the boxed areas (arrowheads), while their relative position is shown at low magnification (arrows). Scale bar, 50 μm, 10 μm in inserts.

(B) Quantification of the data shown in (A). The values indicate mean ± SD of cell counts obtained in three to four mice of each genotype per time point.

(C) Colocalization of progenitor markers PDGFRα (red) and NG2 (green) in the spinal cord of *yy1* cko mice at p18. Scale bar, 20 μm.

$p < 0.0001$). Therefore, we concluded that the ablation of *yy1* in oligodendrocyte progenitors significantly impaired developmental myelination.

The phenotype detected in *yy1* cko mice could be explained by hypothesizing a role for YY1 as transcriptional activator of myelin gene expression. To test this hypothesis, YY1 was overexpressed in the murine oligodendrocyte progenitor cell line Oli-neu (Jung et al., 1995), in the absence or presence of myelin gene promoters driving luciferase reporters. The increased levels of YY1 neither affected the levels of endogenous myelin transcripts nor activated luciferase reporter activity from the transfected myelin promoters (Figure S1). Similar results were obtained in rat-derived CG4 cells when the cells were cultured in the presence of mitogens (data not shown). However, when the cells were cultured in differentiation conditions, YY1 overexpression increased the activity of myelin gene promoters. Interestingly, YY1 increased not only the activity of promoters containing YY1 binding sites (i.e., *plp*; Berndt et al., 2001), but also of myelin gene promoters (i.e., *cgt* and *mbp*) lacking consensus sequence for YY1 (Figure S1). Together these data suggested a more global role for YY1 in regulating myelin gene expression.

Impaired Myelination in *yy1* cko Mice Is Due to Arrested Maturation of Oligodendrocyte Progenitors

A potential role for YY1 in the oligodendrocyte lineage, suggested by the hypomyelinating phenotype of *yy1* cko mice, was a deficit in the maturation of oligodendrocyte progenitors into myelin-forming cells. To test this hypothesis, we conducted a temporal immunophenotypic analysis of oligodendrocyte lineage cells in brain and spinal cord sections of control and mutant mice at postnatal days 2, 11, and 18 (Figure 3 and Figures S2 and S3). These time points were chosen because they define the period of oligodendrocyte differentiation and developmental myelination (Bjelke and Seiger, 1989; Hamano et al., 1998). We used double immunofluorescence with antibodies specific for NG2 to identify progenitors and CC1 to identify mature oligodendrocytes. Confocal microscopy was used for image acquisition, and the relative proportion of $NG2^+$ and $CC1^+$ cells was calculated as a percentage of the total oligodendrocyte lineage population. At p2, a similar proportion of $NG2^+$ progenitors and $CC1^+$ cells was detected in the spinal cord of conditional mutants and control siblings (Figure 3 and Table S1). By p18, however, the oligodendrocyte lineage population of control mice was composed of 60.6% ± 2.9% $CC1^+$ cells while in *yy1*

cko mice only 21.6% ± 1% of the cells were CC1$^+$. Similar differences were observed in the developing corpus callosum (Figure S2). These results were confirmed by immunohistochemistry using antibodies specific for the progenitor marker PDGFRα and for the lipid sulfatide recognized by O4 (Figure S2). Therefore, we concluded that YY1 plays a critical role in modulating the differentiation of progenitors into CC1$^+$ oligodendrocytes.

Because YY1 had been previously reported to negatively regulate p53-induced apoptosis in lymphocytes (Sui et al., 2004; Gronroos et al., 2004), we asked whether the dramatic decrease of CC1$^+$ cells detected in the *yy1* cko mice could have been caused by increased apoptosis. TUNEL assay (Gavrieli et al., 1992) revealed a consistent but statistically insignificant increase of total apoptotic cells in the corpus callosum of *yy1* cko mice compared to control siblings (Figure S2). Similar results were obtained using immunohistochemistry and antibodies specific for the activated form of the apoptosis effector caspase-3 (Gown and Willingham, 2002). At all of the developmental time points analyzed (p2, p11, and p18), we detected a statistically insignificant increase of total apoptotic cells in *yy1* cko compared to sibling controls (Figure S4).

To further define the effect of YY1 deletion on oligodendrocyte differentiation, we performed in vitro ablation experiments. Primary cultures of neonatal cortical oligodendrocyte progenitors, from *yy1*$^{flox/flox}$ mice, were transduced with adenoviral vectors (Gil-Perotin et al., 2006) expressing the recombinase Cre from a CMV promoter (adeno-CMV-*Cre*). Untransduced cultures of *yy1*$^{flox/flox}$ progenitors were used as controls. The efficient excision of *yy1* was confirmed by immunocytochemistry (data not shown). Differentiation was induced by mitogen withdrawal, and the progression along the lineage was followed using stage-specific markers, including immunoreactivity for the late progenitor marker O4 and for the mature oligodendrocyte markers GalC, MBP, and PLP (Figure 4A). The proportion of immunoreactive cells in the YY1$^+$ and YY1$^-$ populations was quantified (Figure 4B). After 3 days in differentiation conditions, more than 45% of control cultures became O4$^+$, and after 2 additional days, a large proportion of the cells displayed the characteristically branched morphology of mature oligodendrocytes and immunoreactivity for myelin proteins. In contrast, in *Cre*-transduced cells, at day 3 only 26% ± 3.1% of cells were characterized by simple morphology and O4$^+$ immunoreactivity, and by day 5 all cells lacked the expression of myelin gene products.

Therefore, ablation of *yy1* in oligodendrocyte progenitors was incompatible with their differentiation into myelin-forming cells.

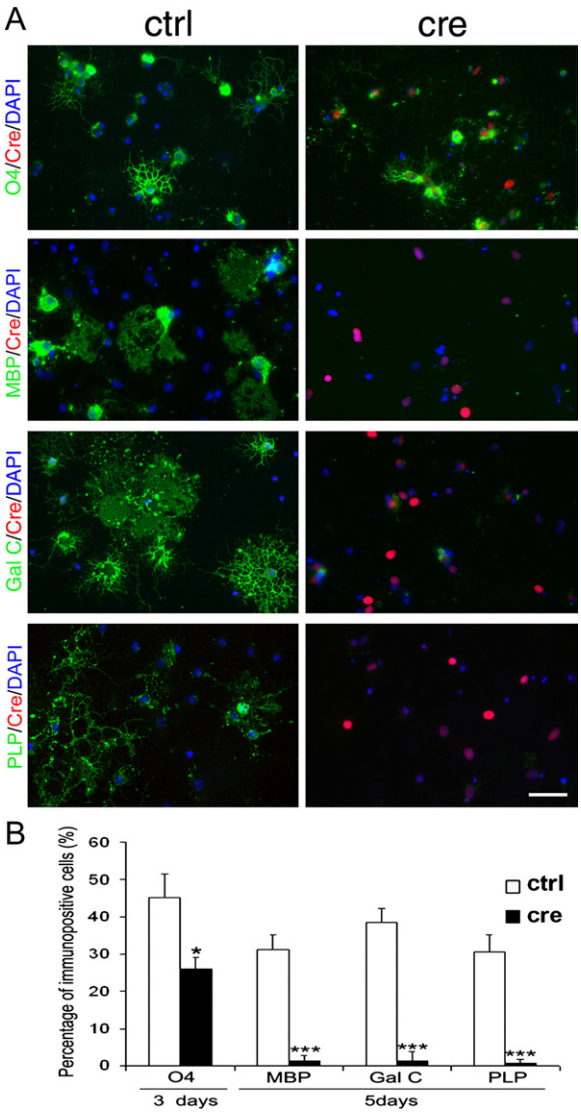

Figure 4. In Vitro Ablation of *yy1* in Oligodendrocyte Progenitors Prevents Their Differentiation into Oligodendrocytes

(A) Primary oligodendrocyte progenitors isolated from the cortex of neonatal *yy1*$^{flox/flox}$ mice were transduced with adenoviral vectors expressing the recombinase Cre (CMV-*Cre*). Forty-eight hours later, the cells were differentiated by mitogen withdrawal. Immunoreactivity for O4 (green) was assessed 3 days later, while immunoreactivity for MBP, GalC, or PLP (green) was assessed 5 days after transduction. Note that the Cre$^+$ *yy1*$^{flox/flox}$ cells (red) progressed to the O4$^+$ stage, but were unable to differentiate into myelin-expressing cells. Scale bar, 50 μm.

(B) Quantification of three distinct experiments performed in duplicate. *p < 0.05, ***p < 0.001. Error bars indicate mean ± SD.

Defective Differentiation in *yy1* cko Mice Is Independent on Impaired Cell Cycle Exit

Since the majority of the cells in *yy1* cko mice retained the progenitor markers NG2 and PDGFRα, it was conceivable that the arrested maturation of these cells was due to their abnormal persistence in the cell cycle. To test this hypothesis, we assessed proliferation and cell cycle exit at several time points of postnatal development in *yy1* cko mice and control siblings (Figure 5). The proliferation rate was inferred by the relative proportion of cells in S phase, calculated after a 1 hr in vivo pulse-labeling with the

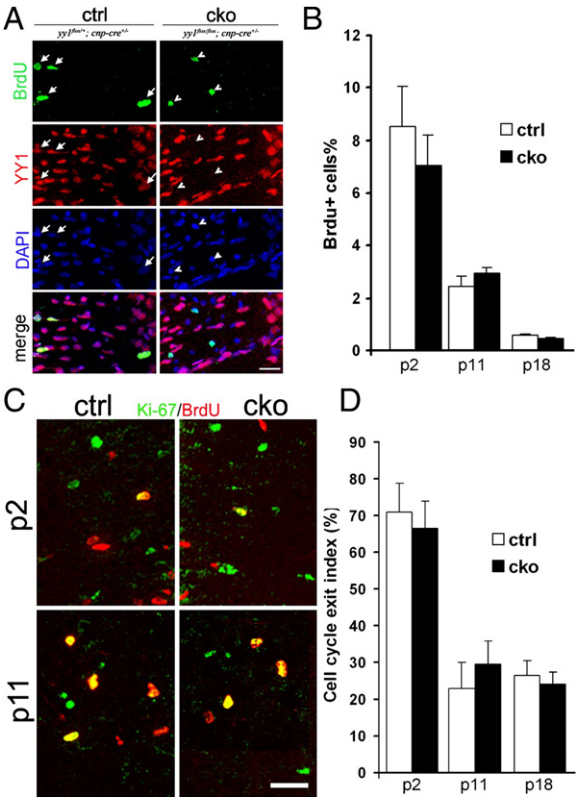

Figure 5. Lack of YY1 Does Not Affect the Ability of Oligodendrocyte Progenitors to Exit from the Cell Cycle

(A) Confocal image of spinal cord sections of p2 *yy1* cko and control siblings (ctrl) in vivo labeled with BrdU for 1 hr. Immunohistochemistry with YY1 (red), BrdU (green), and DAPI (blue) revealed a similar distribution $BrdU^+$ cells in mice of the two genotypes. Scale bar, 20 μm.

(B) Quantification of $BrdU^+$ cells in white matter tracts of the spinal cord at the indicated time points; error bars indicate average cell numbers ± SD. *$p < 0.05$.

(C) Representative images of spinal cord sections of control and cko mice stained with antibodies against Ki-67 (green) to identify cells in any phase of the cell cycle except for G0 and BrdU (red) to identify cells in S phase at the time of labeling.

(D) Cell cycle exit index was calculated by dividing the number of $BrdU^+/Ki\text{-}67^-$ cells by the total number of $BrdU^+$ cells. Error bars indicate mean ± SD.

Scale bar, 20 μm.

thymidine analog bromodeoxyuridine (BrdU). Immunohistochemical analysis of sections of the neonatal spinal cord of *yy1* conditional knockout and control mice revealed a similar progressive reduction of the proportion of BrdU-labeled cells as the animals developed from p2 to p18 (Figures 5A and 5B). Similar results were obtained in primary cultures of $yy1^{flox/flox}$ oligodendrocyte progenitors that were either not transduced or transduced with adeno-CMV-Cre in order to ablate *yy1* in vitro (Figure S5A). Consistently, overexpression of YY1 in immortalized Oli-neu progenitors did not change the proportion of BrdU-labeled cells (Figure S5B) and did not affect the levels of cell cycle regulators (Figure S5C). Together these data suggest that YY1 does not affect cell division and that the $NG2^+$ cells in the *yy1* cko mice were not proliferating.

To further rule out the possibility that oligodendrocyte progenitors were unable to exit from the cell cycle in *yy1* mutant mice, we performed double labeling with BrdU and Ki-67 to calculate the cell cycle exit index (Chenn and Walsh, 2002; Siegenthaler and Miller, 2005). Conditional *yy1* mice and control siblings received a single BrdU injection and were sacrificed 1 hr later. The brains were sectioned and immunostained with antibodies specific for BrdU, to label a cohort of cells in S phase, and for Ki-67, to label proliferating cells throughout the phases of the cell cycle, but not in G0. Double-immunoreactive $BrdU^+/Ki\text{-}67^+$ cells measured the proportion of cycling cells, while the remaining $BrdU^+/Ki\text{-}67^-$ cells indicated cells that had exited from the cell cycle. The cell cycle exit index was calculated by dividing the number of $BrdU^+/Ki\text{-}67^-$ cells by the total population of $BrdU^+$ cells. Our results indicate that at p2, p11, and p18 the cell cycle exit index was similar in cko and control mice (Figures 5C and 5D). From these data we conclude that lack of YY1 does not affect the ability of oligodendrocyte progenitors to exit from the cell cycle. In agreement with the similar apoptotic rate and cell cycle exit index, the total number of cells measured in *yy1* cko mice was similar to the number measured in control siblings, at each of the developmental points analyzed (Table S1).

Thus, the defective differentiation of oligodendrocyte progenitor observed in the knockout mice could not be explained in terms of increased apoptosis or persistent proliferation.

YY1 Repress the Expression of Myelin Gene Transcriptional Inhibitors

To understand how YY1 regulates oligodendrocyte progenitor differentiation, we performed a gene expression profile study of postnatal day 2 cko and control brains, using Affymetrix microarrays. Among the genes whose expression was affected by YY1 deletion, we focused our analysis on those encoding for transcription factors and validated their expression by RT-PCR (Figure 6A) and quantitative RT-PCR (Figure 6B). We detected a 9-fold increase of the transcription factor Tcf4, a 4-fold increase of the differentiation inhibitor Id4, and a 2-fold increase of the HMG-protein Sox11 (Figure 6B). No changes were detected in the expression levels of nestin or REST, which were previously shown to be regulated by YY1 in mouse embryonic fibroblasts (Affar et al., 2006). A repressive role for YY1 on the transcriptional regulation of the transcription factors Tcf4 and Id4 was further supported by overexpression studies in oligodendrocyte progenitors (Figure 6C).

Id4 is a well-characterized inhibitor of oligodendrocyte progenitor differentiation and myelin gene expression (Kondo and Raff, 2000; Samanta and Kessler 2004; Marin-Husstege et al., 2006). Tcf4 is a critical downstream effector of Wnt signaling (Cho and Dressler, 1998; Tetsu and McCormick, 1999), but its role in the oligodendrocyte

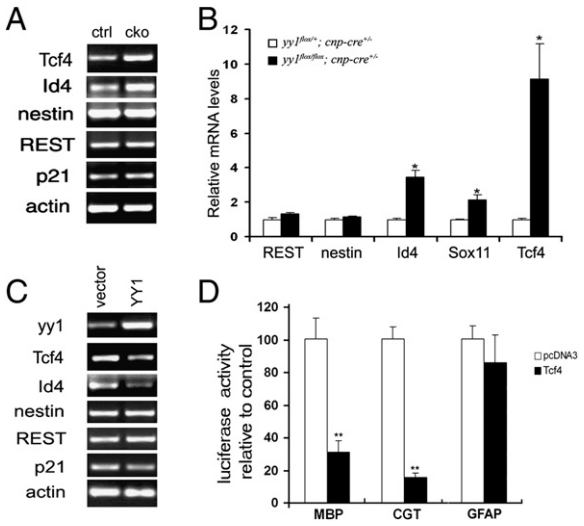

Figure 6. YY1 Is a Repressor of Oligodendrocyte Differentiation Inhibitors Id4 and Tcf4

(A) Validation of gene expression profiling studies in the cortex of *yy1* cko mice and control littermates at p2, by semiquantitative (A) and quantitative (B) RT-PCR.

(B) The bar graphs represent relateive RNA levels that were normalized to the levels of GAPDH, and the values in the control mice were arbitrarily set as 1. Error bars indicate mean ± SD. *$p < 0.05$.

(C) The levels of Tcf4 and Id4 were downregulated in oligodendrocyte progenitors transfected with pCX-*yy1*-EGFP (YY1) and not in cells transfected with pCX-EGFP vector as control.

(D) Bar graphs represent the luciferase activity measured in extracts from immortalized oligodendrocyte progenitors (Oli-neu cells) cotransfected with Tcf4 and either myelin (*mbp*-luc, CGT-luc) or astrocyte-specific (*gfap*-luc) promoters driving luciferase reporters. The luciferase activity in pCDNA3-transfected controls was arbitrarily set as 100; ** $p < 0.01$. The error bars indicate mean luciferase values ± SD.

lineage has not been characterized. To define the functional role of Tcf4 in the oligodendrocyte lineage, we coexpressed this factor with myelin gene promoters (i.e., *mbp*, *cgt*) driving luciferase reporter genes. Cotransfection of Tcf4 with luciferase reporter driven by the astrocyte-specific promoter GFAP was used as control. Interestingly, Tcf4 significantly decreased the activity of myelin gene promoters compared to vector-transfected controls, but did not affect the activity of the *gfap* promoter (Figure 6D).

Together, these data support a role for YY1 in oligodendrocyte progenitor differentiation that is dependent on the downregulation of transcriptional inhibitors of myelin genes.

Lineage-Specific Effect of YY1 in Oligodendrocyte Progenitor Differentiation

The effect of *yy1* ablation on oligodendrocyte progenitor maturation raised the question of the specificity of this transcription factor for the oligodendrocyte lineage. In other words, it was important to define whether the function of YY1 in oligodendrocyte progenitor differentiation was specific or whether YY1 was a transcription factor that is universally required for the differentiation into any neural lineage. We therefore performed in vitro ablation of *yy1* in multipotential nestin$^+$ precursors and then allowed them to differentiate into distinct lineages (Figure 7). Nestin$^+$ precursors were generated from the subventricular zone of neonatal *yy1*$^{flox/flox}$ mice and grown in suspension as neurospheres (Figure 7A). After dissociation, the cells were transduced with adeno-CMV-*Cre*, and *yy1* excision was confirmed by immunocytochemistry (data not shown). Untransduced cultures were used as controls. Differentiation was induced by culturing the cells in a medium permissive for differentiation along distinct lineages (Belachew et al., 2003), and the cells were analyzed by immunocytochemistry, using markers specific for astrocytes (i.e., GFAP), oligodendrocytes (i.e., GalC), and neurons (i.e., TuJ1) (Figure 7A). The number of immunoreactive cells in each lineage that was either YY1$^+$ or YY1$^-$ was counted and expressed as percentage of the total population of cells (Figure 7B). Our results showed a decrease of newly generated oligodendrocytes in cells lacking YY1 but no statistically significant effect on the generation of neurons or astrocytes (Figure 7B). To further evaluate the effect of *yy1* deletion on neuronal differentiation, we repeated the experiment and cultured the cells in conditions promoting neuronal differentiation (i.e., in the presence of retinoic acid; Wang et al., 2005). Also in this case, *yy1* deletion did not significantly affect neurogenesis from neonatal precursor cells (Figures 7C and 7D).

We therefore hypothesized that the lineage-specific role of YY1 in the oligodendrocyte lineage included the transcriptional regulation of the inhibitory transcription factors Tcf4 and Id4. To test this hypothesis, YY1 was overexpressed in progenitors, and the cells were later allowed to differentiate either into oligodendrocytes or into astrocytes. The RNA was isolated and the relative levels of the transcripts were measured by quantitative real-time PCR (Figure 8A). Consistent with a specific role of YY1 in the oligodendrocyte lineage, the levels of Tcf4 and Id4 were decreased only when cells differentiated into oligodendrocytes, not when they differentiated into astrocytes (Figure 8A). To understand the molecular basis of this YY1-dependent lineage-specific repression of *Tcf4* and *Id4*, we performed coimmunoprecipitation with YY1 and HDACs, the chromatin-modifying enzymes involved in transcriptional repression and previously shown to be critical for oligodendrocyte differentiation (Marin-Husstege et al., 2002; Shen et al., 2005). YY1 was immunoprecipitated from protein extracts generated from undifferentiated progenitors and from cells induced to differentiate along the oligodendrocyte lineage. As control for lineage specificity, we also included extracts from cells induced to differentiate into astrocytes by BMP4 treatment. The association was assayed by Western blot, using antibodies specific for HDAC isoforms 1 and 2 (Figure 8B). The association of YY1 with HDAC was weakly detected in undifferentiated progenitors and astrocytes and was enhanced in differentiating oligodendrocytes (Figure 8B). Next, we examined the functional status of YY1 in relation to the differentiative state of progenitors, using YY1

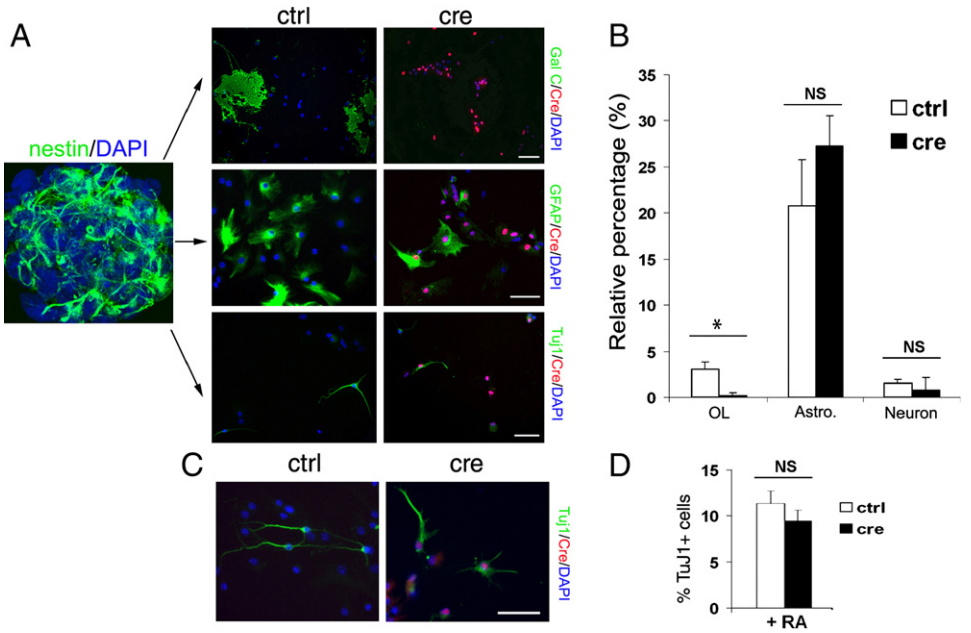

Figure 7. In Vitro Ablation of *yy1* in Nestin$^+$ Precursor Cells Impairs Oligodendrocyte Differentiation in a Cell-Type-Specific Manner

(A) Subventricular zone (SVZ) cells were isolated from neonatal *yy1$^{flox/flox}$* mice and cultured as nestin$^+$ (green) neurospheres. Dissociated neurospheres without (ctrl) or with adenovirus-CMV-Cre transduction (cre) were allowed to differentiate into oligodendrocytes, astrocytes, and neurons. Seven days later, the cultures were stained for Cre (red) and the lineage-specific markers (green): Gal C (oligodendrocytes), GFAP (astrocytes), and Tuj1 (neurons). Scale bar, 50 μm.

(B) Bar graphs indicate the percentage of immunoreactive cells relative to total cell number. *$p < 0.05$. Error bars indicate mean ± SD.

(C) The experiment was repeated in medium supplemented with retinoic acid (RA) to promote neuronal differentiation.

(D) Bar graphs show that even in this culture condition no significant difference was detected between control and *yy1*$^-$ cells. Error bars indicate mean ± SD.

TransLucent Reporter Vector (Panomics, CA). This vector contains a luciferase reporter under the control of an enhancer element containing multiple YY1 binding sites, upstream of the minimal Herpes Simplex thymidine kinase promoter (Dai et al., 2005). After transfection of this reporter vector in undifferentiated progenitors, cells were exposed to mitogenic, astrogliogenic, or oligodendrogliogenic stimuli, and luciferase activity was measured. In differentiating oligodendrocytes, the luciferase activity was 3-fold higher than in astrocytes (Figure 8C). Therefore, the functional ability of YY1 to bind DNA and form protein complexes with HDAC was greater when progenitors differentiated into oligodendrocytes rather than into astrocytes.

We then asked whether HDAC1 was recruited to YY1 binding sites (NNCCATNN; Shrivastava and Calame, 1994; Yant et al., 1995) in the promoter region of *Tcf4* and *Id4* (Figures 8D and 8E). Chromatin immunoprecipitation (ChIP) was performed using antibodies against YY1 or HDAC1, and the DNA was amplified using primers flanking the promoter regions containing YY1 binding sites. Immunoprecipitation without antibodies and amplification with primers in regions lacking YY1 consensus sequences were used as negative controls. We detected increased YY1 and HDAC1 recruitment to the same regions of the *Tcf4* promoter containing YY1 binding sites in differentiating cells compared to undifferentiated cells (Figure 8D). This was not observed when progenitors differentiated into astrocytes (Figure 8D). A similar mechanism was detected for the YY1-dependent regulation of the *Id4* promoter (Figure 8E). Also in this case the enhanced recruitment of HDAC1 to the YY1 binding sites was observed when progenitors differentiated into oligodendrocytes, but not when they differentiated into astrocytes (Figure 8E).

From the combined in vitro and in vivo evidence, YY1 emerges as a lineage-specific modulator of the transcriptional network leading to oligodendrocyte differentiation (Figure 8F).

DISCUSSION

Oligodendrocyte Progenitor Differentiation Is Regulated by Intrinsic and Extrinsic Mechanisms

Myelination of the CNS is dependent on the correct execution of a genetic program of oligodendrocyte differentiation that culminates with the timely and coordinated expression of several myelin genes. It is well established that differentiation of oligodendrocytes from progenitors requires exit from the cell cycle and the initiation of a transcriptional program leading to myelin gene expression (Bogler et al., 1990; Casaccia-Bonnefil and Liu, 2003).

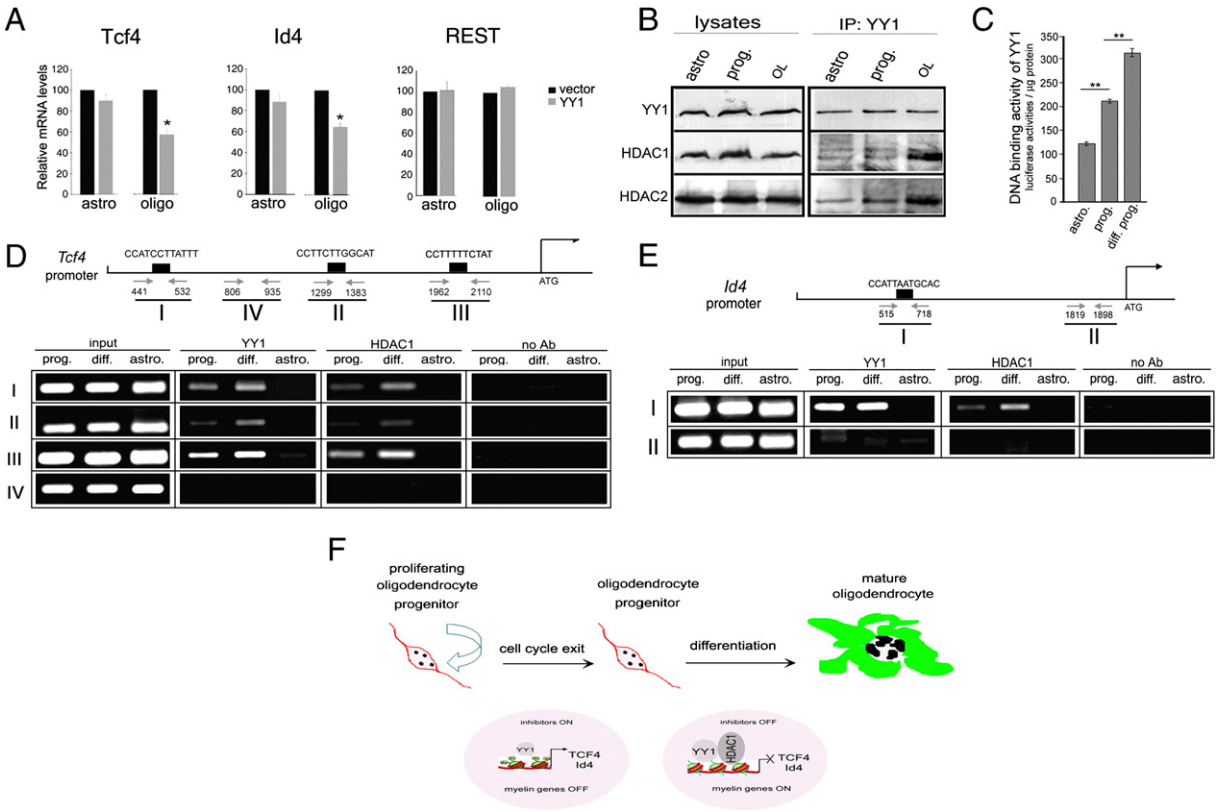

Figure 8. The YY1-Dependent Repression of Tcf4 and Id4 Is Lineage Specific and Mediated by Recruitment of HDAC1 to Their Promoters

(A) The bar graphs indicate the relative mRNA levels of Tcf4, Id4, and REST by quantitative RT-PCR in oligodendrocyte progenitors transfected with pCX-EGFP (vector) or pCX-*yy1*-EGFP (YY1) and induced to differentiate into astrocytes (astro) or oligodendrocytes (oligo). The transcripts in vector-transfected cells were arbitrarily set as 100; *$p < 0.05$. Error bars indicate mean ± SD.

(B) Coimmunoprecipitation of YY1 and HDAC-1 and -2. Western blot analysis of whole-cell lysates (lysates) and YY1 immunoprecipitated protein extracts (IP:YY1) derived from undifferentiated progenitors (prog) and cells differentiated into oligodendrocytes (diff prog) or astrocytes (astro).

(C) YY1 activity, measured by TransLucent vector reporter system. Note the increased activity of YY1 in cultures of progenitors differentiating into oligodendrocytes (diff. prog.) compared to undifferentiated cells (prog.) or cells differentiating into astrocytes (astro). **$p < 0.01$.

(D) Chromatin immunoprecipitation (ChIP) of samples isolated from progenitors (prog), differentiating oligodendrocytes (diff), or astrocytes (astro) and immunoprecipitated with antibodies against YY1 and HDAC1. The diagram shows the *Tcf4* promoter with the relative position of the YY1 consensus sequences (black boxes) and the regions of DNA (roman numerals) amplified by specific primer pairs (arrows). Input DNA was used as positive control, while ChIP in the absence of antibodies or amplification of immunoprecipitated chromatin with primers for region IV were used as negative controls.

(E) ChIP of samples isolated in the same conditions described above. The diagram shows the *Id4* promoter, with the position of the YY1 consensus sequence (black box) and the regions of the promoter (roman numerals) amplified by specific primer pairs (arrows). YY1 was bound to the *Id4* promoter in progenitors, but it recruited HDAC1 only when cells differentiated into oligodendrocytes. No binding was observed in progenitors differentiating into astrocytes.

(F) Model of oligodendrocyte progenitor differentiation as two-step event. First, proliferating progenitors exit from the cell cycle and remain in an undifferentiated state characterized by high levels of transcriptional inhibitors (Id4, Tcf4) and lack of myelin gene expression. As the progenitors begin differentiating, repressive complexes containing YY1 and HDAC1 are recruited to the promoter of these inhibitors. The decreased levels of these inhibitory molecules allow myelin gene expression to begin.

However, the events occurring during this transition remain unidentified. This study identifies a function for the transcription factor YY1 as a molecule regulating the early stages of oligodendrocyte progenitor differentiation after cell cycle exit (Figure 8F).

Original studies in clonal cultures of oligodendrocyte progenitors purified from the optic nerve suggested the existence of a mechanism linking the activation of a differentiation program to exit from the cell cycle (Temple and Raff, 1986; Hart et al., 1989). This concept was supported by the evidence that mitogens prevent oligodendrocyte progenitor differentiation (Gard and Pfeiffer, 1993; Barres et al., 1994; Calver et al., 1998; Nakatsuji and Miller 2001) and that the ability of oligodendrocyte progenitors to timely differentiate is delayed in animals with genetic deletion of cell cycle regulators (Casaccia-Bonnefil et al., 1997, 1999; Durand et al., 1997, 1998). Together, these studies led to a model of lineage progression as

binary state, such that a progenitor could either proliferate while remaining undifferentiated or progress to postmitotic differentiated oligodendrocyte. However, the induction of cell cycle exit was not sufficient, per se, to induce oligodendrocyte progenitor differentiation (Tikoo et al., 1998; Tang et al., 1999). Therefore, "differentiation" was redefined as the linear succession of sequential states, from proliferative and undifferentiated cells to quiescent progenitors to mature oligodendrocytes (Pfeiffer et al., 1993), each defined by a complex balance of positive (i.e., Sox10, Mash1) and negative (i.e., Id4, Hes5) regulators (Gokhan et al., 2005; Liu et al., 2006).

Previous studies from our laboratory defined HDAC activity as necessary during the transition between cell cycle exit and the initiation of a developmental program of differentiation (Marin-Husstege et al., 2002; Shen et al., 2005). In this study, we identify YY1 as the molecule that recruits HDAC to the promoter of transcriptional inhibitors of myelin genes (i.e., Id4, Tcf4). This YY1-mediated repressive event occurs only when progenitors differentiate into oligodendrocytes, not when they become astrocytes.

YY1 Deletion Impairs Oligodendrocyte Progenitor Differentiation without Affecting Cell Cycle Exit

The most prominent feature of in vivo ablation of *yy1* in the oligodendrocyte lineage is the detection of a shaking phenotype characterized by ultrastructural evidence of defective developmental myelination, due to impaired differentiation of oligodendrocyte progenitors. In the spinal cord, small-caliber axons show complete lack of myelin, and a small percentage of large-caliber axons are wrapped by thin myelin sheaths. At the biochemical level, defective myelination in the *yy1* cko mice is associated with lower levels of myelin proteins and transcripts compared to sibling controls and inefficient differentiation of progenitors into myelinating oligodendrocytes.

In the CNS of control mice, progenitors differentiate into myelinating oligodendrocytes during the first 3 weeks of postnatal development. Differentiation can be assessed by detecting a progressive decline in the number of cells expressing progenitor markers and the corresponding increase in the proportion of differentiated cells. In *yy1* mutants, we detect a greater proportion of cells expressing progenitor markers that is paralleled by a smaller proportion of mature oligodendrocytes.

A potential explanation for defective oligodendrocyte progenitor differentiation was the possibility that progenitors were retained in a proliferative state due to lack of *yy1*. However, the ability of progenitors to exit from the cell cycle and the number of cells in S phase are not affected by YY1 levels. In addition, the increased absolute number of progenitors during the first postnatal days, a distinctive feature of defective differentiation due to persistent proliferation (Casaccia-Bonnefil et al., 1997), is not detected in *yy1* cko mice. Rather, the differences between *yy1* cko mice and control siblings are best detected at later developmental time points, coincident with the acquisition of a differentiated phenotype.

Based on this cumulative evidence, we conclude that YY1 modulates oligodendrocyte progenitor differentiation independently of cell division.

YY1-Dependent Pathways Modulating Myelination

We have previously discussed the importance of positive and negative regulators of myelin gene expression in defining the transcriptional network that defines the myelinating phenotype. In this study, the experimental results identify the role of YY1 in oligodendrocyte differentiation as lineage-specific repressor of genes that inhibit myelin gene expression, including the repressive HLH protein Id4 and the HMG protein Tcf4. Higher levels of Id4 and Tcf4 were detected in the brains of *yy1* cko mice compared to control siblings, and lower levels of Id4 and Tcf4 were detected in YY1-overexpressing cells compared to the vector-transfected controls. The role of Id4 as an inhibitor of oligodendrocyte progenitor differentiation and myelin gene expression has been previously described (Kondo and Raff, 2000; Samanta and Kessler, 2004; Marin-Husstege et al., 2006). The role of Tcf4 as inhibitor of oligodendrocyte differentiation is less well defined. Tcf4 is a member of the TCF/Lef family of HMG box transcription factors, and it is highly expressed in the developing central nervous system (Cho and Dressler, 1998; Korinek et al., 1998). Tcf4 is functionally upregulated by the Wnt signaling pathway (Korinek et al., 1998), which has been shown to inhibit oligodendrocyte differentiation (Shimizu et al., 2005) and to be downregulated by the Shh pathway, which favors oligodendrogliogenesis (Ishibashi and McMahon, 2002). Other studies indicated that the levels of Tcf4 are upregulated by the inhibition of HDAC activity (Saegusa et al., 2005; Yamaguchi et al., 2005). Taken together with our observation that Tcf4 represses the promoter activities of myelin genes *mbp* and *cgt*, this identifies Tcf4 as an inhibitor of oligodendrocyte differentiation. Therefore, YY1 acts as repressor of transcriptional inhibitors of the bHLH (i.e., Id4) and HMG (i.e., Tcf4) family and enhances progenitor differentiation and myelin gene expression. This explains also why YY1 overexpression increased the luciferase activity of reporters driven not only by the *plp* promoter (which contains YY1 consensus sequences) but also that of reporters driven by *mbp* or *cgt* promoters (which lack YY1 consensus sequences) when the cultures were exposed to differentiation conditions.

An alternative possibility was recently suggested by the report that YY1 can also regulate the promoter of beta-site amyloid precursor protein-cleaving enzyme 1 (*bace1*) in primary neurons and astrocytes (Nowak et al., 2006). BACE1 has been shown to influence myelination through cleavage of neuregulins (Willem et al., 2006; Hu et al., 2006). Even though the levels of BACE were not downregulated in the *yy1* cko (data not shown), we cannot exclude the possibility that neuronal or astrocytic regulation of BACE1 levels by YY1 could influence myelination in normal development.

From these data, we conclude that the effect of YY1 on myelin gene expression is indirect and is mediated by the recruitment of the chromatin modifier enzyme HDAC to YY1 binding sites in the promoter of transcriptional inhibitors of myelin gene expression.

The Effect of YY1 on Oligodendrocyte Progenitor Differentiation Is Lineage Specific

This study supports a cell-type-specific role of YY1 in the oligodendrocyte lineage. In differentiating myoblasts (Lee and Lee, 1994; Latinkic et al., 2004) and keratinocytes (Xu et al., 2004), YY1 prevents the expression of differentiation genes and is downregulated during differentiation (Walowitz et al., 1998). In contrast, in oligodendrocyte lineage cells, YY1 facilitates differentiation of progenitors, and its expression remains constant throughout development. Importantly, in vitro ablation of YY1 in nestin^{+} precursor cells prevents the generation of oligodendrocytes but not other lineages. Finally, the transcriptional targets of YY1 are not equivalent in distinct cell types. In mouse embryonic fibroblasts, for instance, YY1 has been reported to modulate the expression levels of REST, a repressor of neuronal fate (Affar et al., 2006; Ballas et al., 2005), but this was not detected in our study. The experimental evidence provided in this study supports the role of YY1 as lineage-specific activator of HDAC enzymatic activity during oligodendrocyte progenitor differentiation. This interpretation is based on the association of YY1 with the chromatin modifier enzyme HDAC1 only when progenitors differentiate into oligodendrocytes, not when they differentiate into astrocytes. The lineage-specific formation of YY1/HDAC1 protein complexes was also associated with a 3-fold greater DNA-binding activity of YY1 in progenitors differentiating into oligodendrocytes compared to astrocytes. Finally, these data are consistent with the detection of YY1/HDAC1 repressive complexes in the promoter regions of *Tcf4* and *Id4* only during differentiation of progenitors into oligodendrocytes. Therefore, although previous studies had already reported the constitutive association of YY1 with HDAC1 in HeLa (Yao et al., 2001) and in erythroleukemia cell lines, our study demonstrates an inducible and lineage-specific association between YY1 and HDAC1 in oligodendrocyte progenitors.

These results improve our understanding of oligodendrocyte development by providing a mechanistic insight into the critical transition between cell cycle exit and initiation of a transcriptional program of differentiation.

EXPERIMENTAL PROCEDURES

Animals

All of the mice used in this study were handled according to protocols approved by the Institutional IACUC committee. Conditional *yy1* mutants were generated by crossing *yy1*$^{flox/flox}$ mice (Affar et al., 2006) with *Cnp1-cre* mice (Lappe-Siefke et al., 2003), as detailed in Supplemental Experimental Procedures.

Antibodies and Plasmids

See Supplemental Experimental Procedures.

Immunohistochemistry, Immunocytochemistry, and Image Processing

Cells and sections were stained overnight with primary antibodies as previously described (Marin-Husstege et al., 2006). After incubation with fluorophore-conjugated secondaries, immunoreactivity was analyzed, using the Zeiss LSM 510 Meta confocal laser scanning microscope. Image acquisition and quantification can be found in Supplemental Experimental Procedures.

BrdU Incorporation, Caspase-3 Immunohistochemistry, and TUNEL Assays

Mice received 10 mg/kg BrdU injection 1 hr before sacrifice. After perfusion and cryopreservation, brains were sectioned and stained as previously described (Shen et al., 2005). To identify the apoptotic cells, terminal deoxynucleotidyl transferase-mediated dUTP end labeling (TUNEL) assay and immunohistochemistry using an antibody against activated cleaved 17/19 kD caspase-3 were performed (Supplemental Experimental Procedures).

Electron Microscopy

Yy1 conditional mutant mice and control siblings were deeply anesthetized and transcardially perfused with 0.9% NaCl followed by 0.1 M Millonig's solution containing 4% formaldehyde and 5% glutaraldehyde (pH 7.3). The mice were postfixed for 2 weeks in the same fixative solution at 4°C. Following the postfixation, tissue samples from cervical spinal cord and corpus callosum at the level of the fornix were harvested and processed for standard transmission electron microscopic analysis as previously described (Dupree et al., 1998; Marcus et al., 2006). For quantification details, see Supplemental Experimental Procedures.

Neurosphere Culture of SVZ-Derived Multipotential Progenitors

The lateral SVZ was dissected from *yy1*$^{flox/flox}$ neonatal mice and dissociated with papain. Approximately 1–2 × 10^4 SVZ cells (one brain) per well of a 24-well plate were plated to form neurospheres and grown in the presence of EGF and bFGF. After 7 days, the neurospheres were mechanically dissociated and differentiated for 7 additional days into several lineages (Supplemental Experimental Procedures).

Oligodendrocyte Progenitor Culture

Oligodendrocyte progenitors were isolated from postnatal day 1 cortex of rat or *yy1*$^{flox/flox}$ mice and immunoselected using A2B5 antibodies (for rat cells) and NG2 antibodies (for mouse cells) and secondary antibodies conjugated with magnetic beads (Supplemental Experimental Procedures). Progenitors were cultured in the presence of bFGF and PDGF while oligodendrocyte differentiation was induced by culturing the cells in the absence of mitogens (ODM) and astrocytic differentiation by culturing the cells in BMP4 (100ng/ml). Viral transduction, plasmid transfection protocols, and luciferase assays can be found in Supplemental Experimental Procedures.

Quantitative and Semiquantitative Reverse Transcriptase PCR

Mouse brains or cell pellets were homogenized in Trizol reagent, and RNA was isolated following the manufacturer's instruction. Quantitative real-time PCR was performed using Stratagene SYBR Green PCR master mix in Stratagene MX4000 multiplex quantitative PCR system (Supplemental Experimental Procedures).

Western Blot and Immunoprecipitation

Proteins from the brain were extracted as described in Liu et al. (2006), and immunoprecipitation was conducted using standard biochemical procedures (Supplemental Experimental Procedures).

Chromatin Immunoprecipitation

Chromatin was isolated from 1 × 10^7 oligodendrocyte progenitors (Jung et al., 1995) that were cultured either in growth medium or in oligodendrocyte differentiating medium or were induced to astrocytic

lineage by BMP4 treatment. After immunoprecipitation with 5 μg anti-YY1 antibody (mouse monoclonal sc-7341X, Santa Cruz) or 5 μg of anti-HDAC1 antibody (rabbit polyclonal, Affinity BioReagents, Inc), chromatin was reverse crosslinked using the EZ ChIP kit (Upstate Biotechnology). Proteins were digested with proteinase K, and the recovered DNA was purified using the QIAGEN QIAquick PCR purification kit and subjected to PCR amplification as previously described (Liu et al., 2006). Details are in Supplemental Experimental Procedures.

Statistical Methods

Results are expressed as mean ± standard deviation (SD) and statistically analyzed using two-tailed Student's t tests. $p < 0.05$ was considered to be statistically significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/55/2/217/DC1/.

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REFERENCES

Affar, el.B., Gay, F., Shi, Y., Liu, H., Huarte, M., Wu, S., Collins, T., Li, E., and Shi, Y. (2006). Essential dosage-dependent functions of the transcription factor yin yang 1 in late embryonic development and cell cycle progression. Mol. Cell. Biol. *26*, 3565–3581.

Ballas, N., Grunseich, C., Lu, D.D., Speh, J.C., and Mandel, G. (2005). REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. Cell *121*, 645–657.

Barres, B.A., Lazar, M.A., and Raff, M.C. (1994). A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. Development *120*, 1097–1108.

Belachew, S., Chittajallu, R., Aguirre, A.A., Yuan, X., Kirby, M., Anderson, S., and Gallo, V. (2003). Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. J. Cell Biol. *161*, 169–186.

Berndt, J.A., Kim, J.G., Tosic, M., Kim, C., and Hudson, L.D. (2001). The transcriptional regulator Yin Yang 1 activates the myelin PLP gene. J. Neurochem. *77*, 935–942.

Bjelke, B., and Seiger, A. (1989). Morphological distribution of MBP-like immunoreactivity in the brain during development. Int. J. Dev. Neurosci. *7*, 145–164.

Bogler, O., Wren, D., Barnett, S.C., Land, H., and Noble, M. (1990). Cooperation between two growth factors promotes extended self-renewal and inhibits differentiation of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells. Proc. Natl. Acad. Sci. USA *87*, 6368–6372.

Calver, A.R., Hall, A.C., Yu, W.P., Walsh, F.S., Heath, J.K., Betsholtz, C., and Richardson, W.D. (1998). Oligodendrocyte population dynamics and the role of PDGF in vivo. Neuron *20*, 869–882.

Casaccia-Bonnefil, P., and Liu, A. (2003). Relationship between cell cycle molecules and onset of oligodendrocyte differentiation. J. Neurosci. Res. *72*, 1–11.

Casaccia-Bonnefil, P., Tikoo, R., Kiyokawa, H., Friedrich, V., Jr., Chao, M.V., and Koff, A. (1997). Oligodendrocyte precursor differentiation is perturbed in the absence of the cyclin-dependent kinase inhibitor p27Kip1. Genes Dev. *11*, 2335–2346.

Casaccia-Bonnefil, P., Hardy, R.J., Teng, K.K., Levine, J.M., Koff, A., and Chao, M.V. (1999). Loss of p27Kip1 function results in increased proliferative capacity of oligodendrocyte progenitors but unaltered timing of differentiation. Development *126*, 4027–4037.

Chenn, A., and Walsh, C.A. (2002). Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science *297*, 365–369.

Cho, E.A., and Dressler, G.R. (1998). TCF-4 binds beta-catenin and is expressed in distinct regions of the embryonic brain and limbs. Mech. Dev. *77*, 9–18.

Dai, Y., Rahmani, M., Dent, P., and Grant, S. (2005). Blockade of histone deacetylase inhibitor-induced RelA/p65 acetylation and NF-kappaB activation potentiates apoptosis in leukemia cells through a process mediated by oxidative damage, XIAP downregulation, and c-Jun N-terminal kinase 1 activation. Mol. Cell. Biol. *25*, 5429–5444.

Donohoe, M.E., Zhang, X., McGinnis, L., Biggers, J., Li, E., and Shi, Y. (1999). Targeted disruption of mouse Yin Yang 1 transcription factor results in peri-implantation lethality. Mol. Cell. Biol. *19*, 7237–7244.

Dupree, J.L., Coetzee, T., Suzuki, K., and Popko, B. (1998). Myelin abnormalities in mice deficient in galactocerebroside and sulfatide. J. Neurocytol. *27*, 649–659.

Durand, B., Gao, F.B., and Raff, M. (1997). Accumulation of the cyclin-dependent kinase inhibitor p27/Kip1 and the timing of oligodendrocyte differentiation. EMBO J. *16*, 306–317.

Durand, B., Fero, M.L., Roberts, J.M., and Raff, M.C. (1998). p27Kip1 alters the response of cells to mitogen and is part of a cell-intrinsic timer that arrests the cell cycle and initiates differentiation. Curr. Biol. *8*, 431–440.

Gard, A.L., and Pfeiffer, S.E. (1993). Glial cell mitogens bFGF and PDGF differentially regulate development of O4+GalC- oligodendrocyte progenitors. Dev. Biol. *159*, 618–630.

Gavrieli, Y., Sherman, Y., and Ben-Sasson, S.A. (1992). Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J. Cell Biol. *119*, 493–501.

Ghiani, C., and Gallo, V. (2001). Inhibition of cyclin E-cyclin-dependent kinase 2 complex formation and activity is associated with cell cycle arrest and withdrawal in oligodendrocyte progenitor cells. J. Neurosci. *21*, 1274–1282.

Gil-Perotin, S., Marin-Husstege, M., Li, J., Soriano-Navarro, M., Zindy, F., Roussel, M.F., Garcia-Verdugo, J.M., and Casaccia-Bonnefil, P. (2006). Loss of p53 induces changes in the behavior of subventricular zone cells: implication for the genesis of glial tumors. J. Neurosci. *26*, 1107–1116.

Gokhan, S., Marin-Husstege, M., Yung, S.Y., Fontanez, D., Casaccia-Bonnefil, P., and Mehler, M.F. (2005). Combinatorial profiles of oligodendrocyte-selective classes of transcriptional regulators differentially modulate myelin basic protein gene expression. J. Neurosci. *25*, 8311–8321.

Gown, A.M., and Willingham, M.C. (2002). Improved detection of apoptotic cells in archival paraffin sections: immunohistochemistry using antibodies to cleaved caspase 3. J. Histochem. Cytochem. *50*, 449–454.

Griffiths, I.R. (1996). Myelin mutants: model systems for the study of normal and abnormal myelination. Bioessays *18*, 789–797.

Gronroos, E., Terentiev, A.A., Punga, T., and Ericsson, J. (2004). YY1 inhibits the activation of the p53 tumor suppressor in response to genotoxic stress. Proc. Natl. Acad. Sci. USA *101*, 12165–12170.

Hall, A., Giese, N.A., and Richardson, W.D. (1996). Spinal cord oligodendrocytes develop from ventrally derived progenitor cells that express PDGF alpha-receptors. Development *122*, 4085–4094.

Hamano, K., Takeya, T., Iwasaki, N., Nakayama, J., Ohto, T., and Okada, Y. (1998). A quantitative study of the progress of myelination

in the rat central nervous system, using the immunohistochemical method for proteolipid protein. Brain Res. Dev. Brain Res. *108*, 287–293.

Hart, I.K., Richardson, W.D., Bolsover, S.R., and Raff, M.C. (1989). PDGF and intracellular signaling in the timing of oligodendrocyte differentiation. J. Cell Biol. *109*, 3411–3417.

Hu, X., Hicks, C.W., He, W., Wong, P., Macklin, W.B., Trapp, B.D., and Yan, R. (2006). Bace1 modulates myelination in the central and peripheral nervous system. Nat. Neurosci. *9*, 1520–1525.

Ishibashi, M., and McMahon, A.P. (2002). A sonic hedgehog-dependent signaling relay regulates growth of diencephalic and mesencephalic primordia in the early mouse embryo. Development *129*, 4807–4819.

Jung, M., Kramer, E., Grzenkowski, M., Tang, K., Blakemore, W., Aguzzi, A., Khazaie, K., Chlichlia, K., von Blankenfeld, G., Kettenmann, H., et al. (1995). Lines of murine oligodendroglial precursor cells immortalized by an activated neu tyrosine kinase show distinct degrees of interaction with axons in vitro and in vivo. Eur. J. Neurosci. *7*, 1245–1265.

Kondo, T., and Raff, M. (2000). The Id4 HLH protein and the timing of oligodendrocyte differentiation. EMBO J. *19*, 1998–2007.

Korinek, V., Barker, N., Willert, K., Molenaar, M., Roose, J., Wagenaar, G., Markman, M., Lamers, W., Destree, O., and Clevers, H. (1998). Two members of the Tcf family implicated in Wnt/beta-catenin signaling during embryogenesis in the mouse. Mol. Cell. Biol. *18*, 1248–1256.

Lappe-Siefke, C., Goebbels, S., Gravel, M., Nicksch, E., Lee, J., Braun, P.E., Griffiths, I.R., and Nave, K.A. (2003). Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat. Genet. *33*, 366–374.

Latinkic, B.V., Cooper, B., Smith, S., Kotecha, S., Towers, N., Sparrow, D., and Mohun, T.J. (2004). Transcriptional regulation of the cardiac-specific MLC2 gene during Xenopus embryonic development. Development *131*, 669–679.

Lee, Y.M., and Lee, S.C. (1994). Transcriptional activation of the alpha-1 acid glycoprotein gene by YY1 is mediated by its functional interaction with a negative transcription factor. DNA Cell Biol. *13*, 1029–1036.

Ligon, K.L., Fancy, S.P., Franklin, R.J., and Rowitch, D.H. (2006). Olig gene function in CNS development and disease. Glia *54*, 1–10.

Liu, H.F., and Shi, Y. (2005). Yin Yang 1. In Zinc Finger Proteins: from Atomic Contact to Cellular Function, S. Iuchi and N. Kuldell, eds. (Austin, TX: Landers Biosciences and Kluwer Academic Press), pp. 182–193.

Liu, A., Li, J., Marin-Husstege, M., Kageyama, R., Fan, Y., Gelinas, C., and Casaccia-Bonnefil, P. (2006). A molecular insight of Hes5-dependent inhibition of myelin gene expression: old partners and new players. EMBO J. *25*, 4833–4842.

Marcus, J., Honigbaum, S., Shroff, S., Honke, K., Rosenbluth, J., and Dupree, J.L. (2006). Sulfatide is essential for the maintenance of CNS myelin and axon structure. Glia *53*, 372–381.

Marin-Husstege, M., Muggironi, M., Liu, A., and Casaccia-Bonnefil, P. (2002). Histone deacetylase activity is necessary for oligodendrocyte lineage progression. J. Neurosci. *22*, 10333–10345.

Marin-Husstege, M., He, Y., Li, J., Kondo, T., Sablitzky, F., and Casaccia-Bonnefil, P. (2006). Multiple roles of Id4 in developmental myelination: predicted outcomes and unexpected findings. Glia *54*, 285–296.

Miller, R.H. (2005). Dorsally derived oligodendrocytes come of age. Neuron *45*, 1–3.

Nakatsuji, Y., and Miller, R.H. (2001). Control of oligodendrocyte precursor proliferation mediated by density-dependent cell cycle protein expression. Dev. Neurosci. *23*, 356–363.

Nave, K.A. (1994). Neurological mouse mutants and the genes of myelin. J. Neurosci. Res. *38*, 607–612.

Noll, E., and Miller, R.H. (1993). Oligodendrocyte precursors originate at the ventral ventricular zone dorsal to the ventral midline region in the embryonic rat spinal cord. Development *118*, 563–573.

Nowak, K., Lange-Dohna, C., Zeitschel, U., Gunther, A., Luscher, B., Robitzki, A., Perez-Polo, R., and Rossner, S. (2006). The transcription factor Yin Yang 1 is an activator of BACE1 expression. J. Neurochem. *96*, 1696–1707.

Pfeiffer, S.E., Warrington, A.E., and Bansal, R. (1993). The oligodendrocyte and its many cellular processes. Trends Cell Biol. *3*, 191–197.

Pringle, N.P., and Richardson, W.D. (1993). A singularity of PDGF alpha-receptor expression in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage. Development *117*, 525–533.

Richardson, W.D., Kessaris, N., and Pringle, N. (2006). Oligodendrocyte wars. Nat. Rev. Neurosci. *7*, 11–18.

Saegusa, M., Hashimura, M., Kuwata, T., Hamano, M., and Okayasu, I. (2005). Upregulation of TCF4 expression as a transcriptional target of beta-catenin/p300 complexes during trans-differentiation of endometrial carcinoma cells. Lab. Invest. *85*, 768–779.

Samanta, J., and Kessler, J.A. (2004). Interactions between ID and OLIG proteins mediate the inhibitory effects of BMP4 on oligodendroglial differentiation. Development *131*, 4131–4142.

Seto, E., Shi, Y., and Shenk, T. (1991). YY1 is an initiator sequence-binding protein that directs and activates transcription in vitro. Nature *354*, 241–245.

Shen, S., Li, J., and Casaccia-Bonnefil, P. (2005). Histone modifications affect timing of oligodendrocyte progenitor differentiation in the developing rat brain. J. Cell Biol. *169*, 577–589.

Shimizu, T., Kagawa, T., Wada, T., Muroyama, Y., Takada, S., and Ikenaka, K. (2005). Wnt signaling controls the timing of oligodendrocyte development in the spinal cord. Dev. Biol. *282*, 397–410.

Shrivastava, A., and Calame, K. (1994). An analysis of genes regulated by the multi-functional transcriptional regulator Yin Yang-1. Nucleic Acids Res. *22*, 5151–5155.

Siegenthaler, J.A., and Miller, M.W. (2005). Transforming growth factor beta 1 promotes cell cycle exit through the cyclin-dependent kinase inhibitor p21 in the developing cerebral cortex. J. Neurosci. *25*, 8627–8636.

Stolt, C.C., Rehberg, S., Ader, M., Lommes, P., Riethmacher, D., Schachner, M., Bartsch, U., and Wegner, M. (2002). Terminal differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10. Genes Dev. *16*, 165–170.

Sui, G., Affar el, B., Shi, Y., Brignone, C., Wall, N.R., Yin, P., Donohoe, M., Luke, M.P., Calvo, D., Grossman, S.R., and Shi, Y. (2004). Yin Yang 1 is a negative regulator of p53. Cell *117*, 859–872.

Tang, X.M., Beesley, J.S., Grinspan, J.B., Seth, P., Kamholz, J., and Cambi, F. (1999). Cell cycle arrest induced by ectopic expression of p27 is not sufficient to promote oligodendrocyte differentiation. J. Cell. Biochem. *76*, 270–279.

Temple, S., and Raff, M.C. (1986). Clonal analysis of oligodendrocyte development in culture: evidence for a developmental clock that counts cell divisions. Cell *44*, 773–779.

Tetsu, O., and McCormick, F. (1999). Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature *398*, 422–426.

Thomas, M.J., and Seto, E. (1999). Unlocking the mechanisms of transcription factor YY1: are chromatin modifying enzymes the key? Gene *236*, 197–208.

Tikoo, R., Osterhout, D.J., Casaccia-Bonnefil, P., Seth, P., Koff, A., and Chao, M.V. (1998). Ectopic expression of p27Kip1 in oligodendrocyte progenitor cells results in cell-cycle growth arrest. J. Neurobiol. *36*, 431–440.

Walowitz, J.L., Bradley, M.E., Chen, S., and Lee, T. (1998). Proteolytic regulation of the zinc finger transcription factor YY1, a repressor of muscle-restricted gene expression. J. Biol. Chem. *273*, 6656–6661.

Wang, T.W., Zhang, H., and Parent, J.M. (2005). Retinoic acid regulates postnatal neurogenesis in the murine subventricular zone-olfactory bulb pathway. Development *132*, 2721–2732.

Wegner, M. (2000). Transcriptional control in myelinating glia: flavors and spices. Glia *31*, 1–14.

Willem, M., Garratt, A.N., Novak, B., Citron, M., Kaufmann, S., Rittger, A., DeStrooper, B., Saftig, P., Birchmeier, C., and Haass, C. (2006). Control of peripheral nerve myelination by the beta-secretase BACE1. Science *314*, 664–666.

Xu, X., Kawachi, Y., Nakamura, Y., Sakurai, H., Hirota, A., Banno, T., Takahashi, T., Roop, D.R., and Otsuka, F. (2004). Yin-yang 1 negatively regulates the differentiation-specific transcription of mouse loricrin gene in undifferentiated keratinocytes. J. Invest. Dermatol. *123*, 1120–1126.

Yamaguchi, M., Tonou-Fujimori, N., Komori, A., Maeda, R., Nojima, Y., Li, H., Okamoto, H., and Masai, I. (2005). Histone deacetylase 1 regulates retinal neurogenesis in zebrafish by suppressing Wnt and Notch signaling pathways. Development *132*, 3027–3043.

Yant, S.R., Zhu, W., Millinoff, D., Slightom, J.L., Goodman, M., and Gumucio, D.L. (1995). High affinity YY1 binding motifs: identification of two core types (ACAT and CCAT) and distribution of potential binding sites within the human beta globin cluster. Nucleic Acids Res. *23*, 4353–4362.

Yao, Y.L., Yang, W.M., and Seto, E. (2001). Regulation of transcription factor YY1 by acetylation and deacetylation. Mol. Cell. Biol. *21*, 5979–5991.